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A GENERAL OVERVIEW ON BIOSURFACTANT AND ITS PROMISING STRATEGIES IN ENVIRONMENTAL BIOREMEDIATION

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Abstract

Heavy metal contamination from process industries, hydrocarbons from refinery industries and automobile exhaust led to numerous health hazards. Lack of proper waste treatment technologies increases thepollution level in environment.Heavy metal and polyaromatic hydrocarbon pollution had been the main threat for human beings by causing deadly disease "cancer". They get accumulated in environment, consumed and biomagnified causing wide range of health hazards in mammals.Chemical and physical methods of treatment were not efficient and had many drawbacks. The most eco-friendly and conventional way of treating waste is by the use of Biosurfactants. They being amphiphillic can absorb and cleave the fatty acid chains.Biosurfactants has eminent applications in industriesincluding pharmaceutical, cosmetics, food and petroleum industries. Biosurfactants as a remediator has sought many applications since 1960. This review article gives a brief account on classification, screening techniques, production, purification and application of biosurfactant in environmental bioremediations

Keywords: *waste treatment, pollution, biosurfactants, heavy metals, polyaromatic hydrocarbon*

1. Introduction

Microorganisms produce a diverse group of surface-active substances called Biosurfactants. It contain two groups- polar and non polar group, hydrophilic and hydrophobic respectively making them amphiphilic. The hydrophilic-lipophilic balance specifies the surface activity makes surfactants a good foaming, emulsifying and dispersing agent [1]. Biosurfactants are more eco-friendly and less toxic making it a better remediator. Hydrocarbons and few metals serve as a carbon source for microorganisms, but not all can utilise this due to hydrophobicity and floatation properties. Only microorganisms with special features of oil degradation, hydrophobicity and floatation properties are used for biosurfactant production [2]. Biosurfactants decreases the free energy of a system by alternating the bulk molecules at higher energy to interface. The common characteristics of biosurfactants are surface tension reduction,solubility enhancement and low critical micelle concentrations. In between the two immiscible phases, an interfacial boundary lies. The hydrophobic part concentrates at the surface where as the hydrophilic part orients towards the solution [3]. In other words the role of biosurfactants is to enable the growth of microorganism to grow on water immiscible substrates. Biosurfactants does this by reducing the surface tension near the phase boundary so as to make substrate utilisation and metabolism happen [4].The main source of poly aromatic hydrocarbons (PAH) pollution is incomplete combustion of coal, petroleum products. PAHs are also used in the manufacture of pesticides, paints, detergents and dyes. Once it is intoxicated in body, it gets localized in body fats. The metabolism of PAH is carried out by the cytochrome P450- mediated function with mixed oxidase system, first step being oxidation or hydroxidation. This results in formation of epoxides or phenols. Most of the PAH contain a K-

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region and bay-region which on metabolism forms bay- and K- region epoxides which are found to cause cancer [5]. Metals like lead, cadmium, arsenic and mercury are considered to be most toxic metals to human. The levels of these heavy metals are been constantly monitored by WHO. The levels of Cadmium increased from the 20th century due to the abundant use of rechargeable Nickel-Cadmium batteries and lack of proper disposals. Mercury is a major pollutant in industries waste water. The aquatic animals are the food borne barriers of mercury. Combustion of petrol and usage of glazed food containers are the modes where lead enters the human body as toxin. Arsenic contamination is seen in drinking water. The higherlevel exposures of these heavy metals are causing cancer to humans[6]. There are many technologies available for treating hydrocarbon and heavy metals. When compare to other methods bioremediation using biosurfactant is one of the promising treat these pollutants.

2. Classification of biosurfactants

Most biosurfactants are anionic or neutral, whereas some of them are cationic because they contain amine groups [7]. Anionic surfactants are negative due to the presence of sulphonate or sulphur group, they lack ionic constituents and majority of them are polymerisation products of 1, 2-epoxyethane. Cationic biosurfactants gets the positive charge by the presence of quarternary ammonium group [8]. They can also be categorized as low molecular mass and high molecular mass biosurfactant. Lipopeptides, glycolipids,and phospholipids come under the low molecular mass biosurfactant which are effective in reducing surface tensions. Lipopolysaccharides, proteins,lipoproteins,amphipathic polysaccharides, and some complex mixtures of these biopolymers come under high molecular mass biosurfactants; they are effective stabilizers in oil-in-water emulsions[9].

2.1 Glycolipids

Glycolipids are made up of sugars and lipids where the carbohydrate moiety is attached to a fatty acid moiety such as aliphatic acids or hydroxyl aliphatic acids [10]. Glycolipids are the best known biosurfactant for over decades. Carbohydrates like Glucose, galactose, mannose, rhamnose, glucuronic acid and galactose sulphates are its constituents. The fatty acid composition phospholipids and glycolipids have the same composition if produced by same organism [8]. The degree of polarity depends on the substrate hydrocarbon composition [7]. Rhamnolipids, Trehalolipids and Sophorolipids are the classes of glycolipids.

2.1.1 Rhamnolipids

Rhamnolipids are well-known produced by the Pseudomonas aeruginosa [8]. Rhamnolipids contain rhamnose molecules that are linked with one or more β – hydroxydecanoic acid molecule. The OH group of one of the hydroxydecanoic acid is involved in ester formation and another OH group of another hydroxydecanoic acid is implicated in glycosidic linkage of rhamnose disaccharide at the reducing end [11]. Condensation of two molecules of rhamnose sugar and an acetal group that links to the hydrophobic group; forms disaccharide rhamnolipids.Rhamnolipids are used in enhanced dispersion and degradation of different types of hydrocarbons such as vegetable oils and emulsifying hydrocarbons and also in removal of heavy metals from soil [9].

2.1.2 Trehalose lipids

Trehalose lipids are the fundamental component of the cell glycolipids in *Mycobacteria* and *Corynebacteria*. The 2 glucose units of trehalose are linked in an α,α- 1,1 –glycosidic linkage. It is a non- reducing disaccharide [11]. The presence of trehalose esters on the cell surface of *Mycocterium* is due to the presence of serpentine group of Trehalolipids. In the majority of the

species like *Corynebacterium, Norcardia* and *Mycobacterium*disaccharide trehalose linked at C-6 and C6' is associated to mycolic acid. Mycolic acids are long α-branched-β-hydroxy fatty acids that influence the structure and size of the biosurfactant [8].

2.1.3 Sophorolipids

Yeast such as Torulopsis bombicola, T. petrophilum and T. apicola produced sophorolipids. They are dimeric carbohydrates that are linked with long chain hydroxy fatty acids and six to nine different hydrophobic sophorosides. They are not an effective emulsifier though they can lower the surface and interfacial tension [11]. They are majorly produced by Torulopsis sp. Sophorolipids consist of a dimeric sophorose and a hydroxyl fatty acid linked by a β – glycosidic bond. There are two types of sophorolipids, lactonic and non-lactonic (acidic). In which, sophorolipids have an attractive application in cosmetic industries [8].

2.2 Fatty acids, phospholipids, neutral lipids

Corynomycolic acid, Spiculisporic acid and Phosphatidylethanolamine are the sub classes [9]. Fatty acids are produced as a result of microbial oxidation from alkanes. Along with straight chain acids, alkyl branches and fatty acids containing OH groups are also produced by microorganisms. The most active saturated fatty acid ranges are of C12 - C14, which is used in lowering surface and interfacial tensions [8]. Examples are gramicidin and polymixin, they are components of cell structures which have surface activity [10]. Corynomycolic acids are produced by *Corynebacterium lepusis* used in enhancement of bitumen recovery; Spiculisporic acid produced by *Penicillium spiculisporum* is used in metal removal from aqueous solutions; preparation of superfine microcapsules and new emulsion-type organogels [9].

2.3 Lipopeptides

Lipopetides are made up of amino acid chain that is linked to fatty acid, the amino acid chain may be linear or cyclic and the fatty acid chain consists of 13-16 carbon atoms and might be branched [10].Surfactin and Lichenysin are the classes of lipopeptides.Common lipopeptide is Surfactin produced by Bacillus sp. which reduces surface tension from 72 to 27 mN/m that makes it a better surfactant. Surfactin has the capacity to lyse the mammalian erythrocytes and forms spheroplasts [8]. Surfactins are used in hydrocarbon and chlorinated pesticide degradation; heavy metal removal and in phytoextraction.Lichenysin is yet another type of lipopeptide used in enhanced oil recovery [9]. Several biosurfactants are produced by *Bacillus licheniformis* which acts synergistically and reveals good pH, temperature and NaCl stability. A structural and physio-chemical property of lichenysin is similar to surfactin biosurfactant. The surfactants from *Bacillus licheniformis* are capable of lowering the surface tension of water to 27 mN/m and the interfacial tension between n-hexadecane and water to 0.36 mN/m.

2.4 Polymeric biosurfactants

They contain polysaccharide backbone to which fatty acid side chains covalently bind, the intensive classes are Emulsan, alasan and liposan [10]. Emulsan is a d-galactosamine containing polyanionic polysaccharide it is initial released from cell surface as protein complex A polymer named apoemulsan is formed after the removal of the protein [12]. Emulsan was produced from Acinetobacter calcoaceticus, liposan is produced from Candida lipolytica and mannoproteins are synthesised from Saccharomyces cerevisiae. Other polymeric biosurfactants are biodispersan, alasan, food emulsifiers, protein complexes and insecticide emulsifiers [8]. Emulsan, liposan, mannoprotein and alasan are used in stabilisation of hydrocarbon in water emulsions. Biodispersan is used in dispersion of limestone in water [9].

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Bacteria had been used to produce biosurfactants since decades. In few reaches, fungi had like Ulva lactuca, Candida lipolytica and Aspergillus ustus were used [13–15]. The lipophillic part can be long chain fatty acids, hydroxyl fatty acids or α-alkyl-α-hydroxy fatty acids and hydrophilic part can be carbohydrate, amino acid, cyclic peptide, phosphate, carboxylic acid or alcohol [3].A brief classification is shown in Table 1. [4,8]

3. Properties of biosurfactants

3.1 Emulsification index

In the process of emulsification, emulsion is formed. Emulsion is a liquid that contains very small droplets of oil or fat suspended in water [10].Emulsification index is determined using olive oil, crude oil, diesel oil and kerosene oil. Equal volume of surfactant and each of the oil is taken in a test tube and agitated vigorously for 5 min and kept undisturbed.

$$E_{24} = \frac{\text{Height of the emulsion layer (mm)}}{\text{Total height of the liquid (mm)}} \times 100$$

3.2 Surface Tension

The strain was inoculated in MSM medium and kept in shaker at 120 rpm at 35°C and sampled every four hour to examine the mycelia concentration. The supernatant was separated to remove the biomass, and surface tension measurements were made with automated tensiometer by ring method [26, 13]. An efficient surfactant reduces the surface tension of water from 72mN/m to less than 30mN/m [17]. Methods like Direct Surface/Interfacial Tension Measurements, Du-Nouy-Ring Method, Stalagmometric Method, Pendant Drop Shape Technique, Axisymmetric Drop Shape Analysis by Profile were also reported to estimate biosurfactant [18].

Table 1 Microbial source for biosurfactant and their composition.

Organism	Biosurfactant	Composition	
		Lipophillic part	**Hydrophllic part**
Mycobacterium fortuitum	Trehalose lipids	C_{20} or C_{22} Fatty acid	9aa **(3** &al; **2** LThr; tAla; LPro; 2 MeLeu)
Mycobacterium paratubercufosis	Trehalose lipids	C_{20} Fatty acid	3aa Wne, DPhe, Ala, L:eu, Llle)
Nocardia asteroides	Fatty acids, trehalose	B-OH C_{20} Fatty acid	7aa (2 LThr, LPro, LAla, LVal, DAla, oallolle)
Corynebacterium lepus;	Trehalose lipids, glycolipids, fatty acids	C_{13}-C_{24} Fatty acid (25%) Corynomycolic acid (75%)	13 different aa
Streptomyces canus *Streptomyces violaceus*	Streptofactin, Omithine, lysine peptides	3-ai $C_{13,}$ 3-i C_{12} Fatty acid	10 aa

Serratia marcescens	Rhamnolipids, glycolipids	2 β-OH C_{10} FA	2 LSer
Pseudomonas fluorescens *Pseudomonas viscose*	Viscosin, rhamnolipids, lipopolysaccharides	β-OH -C_{10}	LLeu-DGlu Dallo Thr DVal- Llle DSer-LLeu-DSer –Lleu
Pseudomonas rubescens *Thiobacillus thiooxidans* *Rhodopseudomonas spheroides* *Streptomyces sioyaensis*	Lipopolysaccharides, omithine, lysine peptides,sulfonylipids Polyol lipids Streptofactin, omithine, lysine peptides	β-OH Fatty acid	Orn
Agrobacterium temefaceins		β-OH FA	Lys
Gluconobacter cerinus	omithine, lysine peptides	β-OH FA	Orn and Taurine
Candida petrophilum	Sophorolipids, lipopolysaccharides	non-identified FA	Peptide (Asp,Leu Glu and Ala)

3.3 Critical micelle concentration

Critical micelle concentrationis one of the important characteristic of biosurfactant, defined as surface concentration requisite for forming a micelle [13]. Only then surfactant can engulf the target molecule. Above in which no moredrop in surface or interfacial tension was seen[17]. The relationship between surface tension and critical micelle concentration was measured using tensiometer. The surface tension reduced till the CMC remained constant [19].

3.4 Surfactant stability

Surfactant stability was identified by surface tension changes under various environments. The separated surfactant is dissolved in deionised water at a concentration of 500 mg/L. Stability was examined by varying pH, temperature and NaCl concentrations (1-12% w/v)[13]. The cell free broth of *Pseudomonas aeruginosa* was subjected to 70, 100, 121 ^{0}C and also tolower temperature 0-4 ^{0}C. Emulsification index was determined[20].It was reportedthat *Bacillus licheniformis* produced surfactant were not affected by temperature upto 50 ^{0}C and pH in range of 4.5 to 9.0. *B. subtilis*biosurfactant were also stable above 121 0C, pH 5 to 11 and NaCl concentrations up to 20%[17].

3.5 Hydrophobicity index

Bacterial adhesion test to hydrocarbon or BATH assay by vigorously shaking 4 ml of bacterial suspension with 1 ml of n-hexa-decane for 2 min. After the phases were separated, 0.1 ml of aqueous phase was sampled in epifluroscence spectroscopy to find the amount of cells [21]. Hydrophobic interaction chromatography is another method described by [22]. The

bacterial culture (10^7cells/ml) was run through a column with any hydrophobic gel. Using PBS, the samples were eluted and the cells were determined in epifluroscence spectrometer. Particle elecrophoretic measurement is yet another method, were a apparatus named Rank Mark II particle microelectrophoresis is used to determine the mobility of the bacterial cell. The apparatus consist of a glass cell and black platinum electrodes [23].

4. Mechanism of Biosurfactant action

On release of biosurfactant, their monomers organize into micelles so that the hydrophobic portion is aligned towards the center and hydrophilic portion making an interface with water. This alignment of biosurfactant reduces surface tension between water and oil and contributes in micelle formation, increasing the substrate utilisation and oxygen to bacteria enhancing hydrocarbon degradation. Biosurfactants may cause modifications in the cell membrane such as changes in protein composition and cell wall hydrophobicity to promote microbial accessibility to the hydrocarbons. The efficiency of the biosurfactant is determined by the critical micelle concentration, the hydrophilic end of the micelle in biosurfactant that makes an interface with water, s-double, compact, electric layer that would surround the external surface of the micelle sphere is called Stern layer [24]. In case of bacterial decomposition of hydrocarbons, biosurfactants produced by microbes solubilizes oil droplets into the aqueous phase which makes oil uptake much easier by microbes as shown in figure 1.

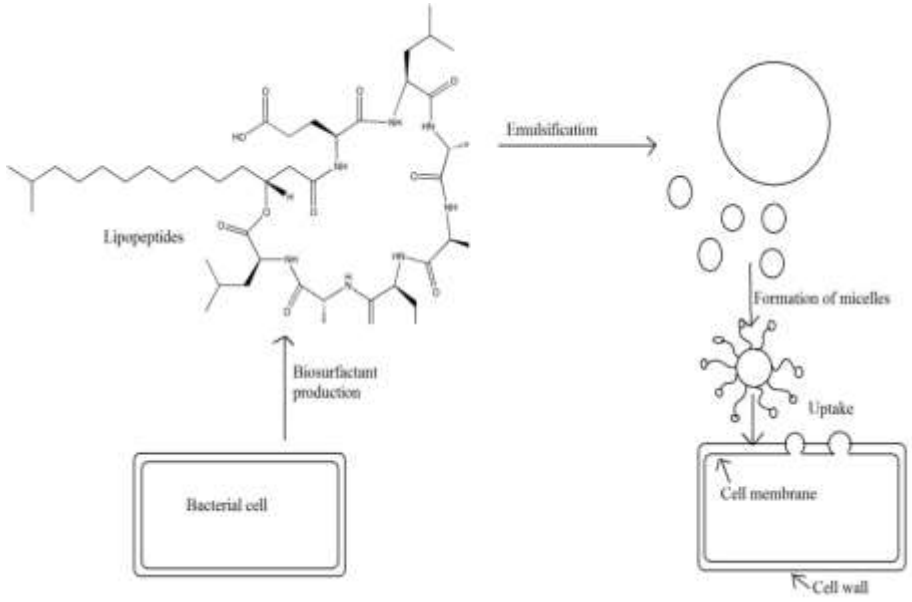

Fig.1: Mechanism of action of biosurfactant

5. Advantages of biosurfactants

Biosurfactants have many advantages over chemical surfactants such as low toxicity, compatibility with human skin, highly biodegradable and low irritancy. Therefore the biosurfactants are better compared to synthetic surfactant. Since chemically synthesised surfactants are petroleum bases, they cause so much environmental hazards and bioaccumulation in living beings. The increasing awareness towards environment needed an alternative source for remediation and that led to the biosurfactant production using microorganisms [25]. Some advantages are discussed as follows.

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5.1 Biodegradability

Biodegradability is one of the important problems regarding environmental pollutants, whereas biosurfactants are biodegradable and being able to break down by natural process with the help of microorganism into simpler components. The digested pollutant is further utilised by the microorganism, it can be suited for bioremediation application and for remediating the oil spilled sites [26].

5.2 Low toxicity

In Biosurfactant, toxicity is very low and hence it doesn't cause any serious damage to the biotic ecosystem. On other hand, chemically synthesised surfactants are toxic to environment and humans making them less usable in industries. Biosurfactants are of biological origin and composed of a simple chemical structure. Thus they easily degrade the pollutants without producing any harmful by-products [25]. Very few cases regarding toxicity were reported against microbial surfactants. According to a report, a synthetic surfactant called Corexit which is anionic had LC50 (concentration lethal to 50% of species tested) against Photobacterium phosphoreum which is ten times lower than rhamnolipid. Hence, chemical surfactants are considered to be highly toxic. The author compared the toxicity test for six biosurfactants, two commercial dispersant and four chemical surfactants, it was observed that most of the biosurfactants are degraded faster except synthetic sucrose-stearate, a type of glycolipid. The degradation rate was much higher in biosurfactants than in chemical surfactants [27]. Pseudomonas aeruginosa produced biosurfactant was compared to a synthetic surfactant called Marlon A-350 that is widely utilized in industries. The chemical surfactant was both toxic and mutagenic whereas biosurfactant was non-toxic and non-mutagenic [28].

5.3 Biocompatibility and digestibility

Biosurfactants have good biocompatibility and digestibility since they are of biological origin. They have wide usage in cosmetic, food, pharmaceutical and agricultural industry. Surfactins, microbial derived biosurfactant can reduce surface and interfacial tension of water to 25 mN/m and <1 mN/ m [25]. On interaction with living organisms, they do not alter their bioactivity and it was well tolerated by all living organisms [29].

5.4 Availability of the raw materials

The production of Biosurfactants can be achieved by very cheap raw materials such as oil wastes, potato process effluent, cassava flour waste water and whey wastes. Carbon sources such as hydrocarbon, carbohydrates, lipids can be used separately or combination of each other for biosurfactant production. Surfactants produced from industrial wastes and by products are of economical benefit and advantageous for bulk production [25].

5.5 Production economics

Based on the application, the cheap raw materials (industrial waste and by products) are used for the biosurfactant production. This is of great interest for bulk production mainly in petroleum based industries.

5.6 Activity at extreme conditions

Biosurfactants are active even at extreme temperature, salinity and pH. Biosurfactants have specific functional groups and are often specific in action [25].

5.7 High specificity

The complex organic molecules with specific functional groups of biosurfactants enable them to be specific in their action. This might be of specific interest in environmental pollution, pharmaceutical products and in cosmetics.

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6. Disadvantages of Biosurfactants

Certain disadvantages are also associated with biosurfactant production. Large scale biosurfactant production is expensive. However, utilization of waste substrates could resolve this issue. Purification of biosurfactants is another major problem especially in food, cosmetic and pharmaceutical industries. Downstream process involves many consecutive steps that are little complex process. Hence high yield of biosurfactant production in bioreactors are essential for their easier recovery and purification. On the other side, high foam formation also retards productivity of biosurfactants [25].

7. Production of Biosurfactants

7.1 Sampling and Isolation of biosurfactant producers

Lin *et al.*isolated microbial strains capable of producing biosurfactant from soil samples contaminated with petroleum using the diesel as sole carbon source. The biosurfactant producing strains were isolated using screening methods [2]. Thavasi and Jeyalakshmi reported that *Bacillus megaterium* was isolated from sea water sample using crude oil as sole carbon source [30]. Ilori *et al,*isolated bacterial strain *Aeromonos* sp.capable of producing the biosurfactant which can degrade oil using Minimal salt medium supplemented with crude oil as sole carbon source [31].

7.2 Screening method

Oil spreading test is used to measure the clear zones formed by a single drop of biosurfactant placed on an oil-water surface [17]. Clearance of zones happens due to utilization of oil by degraders.

CTAB agar plate method is one of a semi-quantitative test for determining extra cellular glycolipids or anionic surfactants. The colonies form dark blue halos on secretion of anionic surfactants due to insoluble ion pair with CTAB and methlylene blue.

Blood agar lysis is a preliminary screening for surfactant production because biosurfactants cause lysis of erythrocytes [18]. 50µl of culture is spot inoculated on blood agar for 48 hrs at 37 ^{o}C, the plated were visually observed for zone clearance. The diameter of zone is a qualitative method for biosurfactant produced[17]. Sheep blood is usually used for the test, and the diameter of the zone increases linearly with increase in concentration of surfactant[32].

Oil spreading assay, where 10µl of crude oil is added to 40 ml distilled water on a petri plate forming a thin oil layer following 10µl of inoculum placed at centre. If there is production of biosurfactant, zone clearance is seen [18]. The diameter of the zone is directly proportional to the surfactant produced, but when surface tension was compared, an inverse linear relationship was obtained [32].

Penetration assay is one of the high throughput assays, its principle being two insoluble phases leads to colour change on contact. Microplate wells are filled with 150µl of hydrophobic paste and covered with 10µl of oil. The culture supernatant is coloured with red stain in ratio of 1:9 which is kept over the paste. If there is the presence of biosurfactant, red colour is seen due to penetration of surfactant degrading the oil layer [18].

Fatty acid composition is analysed by GC-FID, analysis were carried out in GC equipped with capillary column, Helium being the carrier gas. By comparing with the chromatogram of standard fatty acid methyl mixture, the chromatographic peaks were identified[16].

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7.3 Production parameters

7.3.1 Effect of carbon source

Carbon source in a medium plays an important role in a culture medium changing the substrate would alter the structure and properties of the surfactant. The preference of carbon source was decided through its application, for example trehalose lipids productions werealtered by sucrose lipids when sucrose is used as substrate using *Anthrobacter* strain [4]. Carbon source can be categorized into carbohydrates, vegetable oil and hydrocarbons, similarly miscible and non miscible carbon source [10].*Aeromonas sp* produces higher yield of biosurfactantin crude oil, diesel oil and hexadecane, moderate productionin n-tetradecane, kerosene and n-decane and production is low in hexane, toluene, cylcohexane and n-hexane [31]. *Pseudomonas aeruginosa*produces higher biosurfantant in succinate, C_{11} and C_{12} alkanes, pyruvate, fructose, citrate, glycerol, glucose, olive oil and mannitol were used as carbon sources [3].

7.3.2 Effect of nitrogen source

The nitrogen source is also reported to have major influence in biosurfactant production. The best source of nitrogen in rhamnolipid production by *Peudomonas* and *Rhodocuccus* was nitrate. This increased the production till nitrogen starvation period. Sodium nitrate is one of the best nitrogen source for production of rhamnolipids from *P. aeroginosa*[10].*Aeromonas sp.*had maximum emulsification index of 70% when soybean is used as nitrogen source, also peptone gave 68% emulsification index [31].

7.3.3 Effect of C/N ratio

Carbon and nitrogen play very important role in biosurfactant production. Similarly, their composition ratios also play a vital role. Maximum production (13.5 g/l) was observed in rhamnolipids when grown in carbon: nitrogen from 16:1 to 18:1 and production was low in 11:1 [10]. In the growth of *Cunninghamella echinulate* a ratio 4:3 corn steep liquor and soybean oil were used [33]. De, Sorav *et al.* reported that increased C/N ratio, resulted higher rhamnolipids production [25].

7.3.4 Effect of temperature

Higher production of biosurfactants was observed in the temperature range of 28 to 45 ^{0}C using culture medium of*Cunninghamella echinulata*[33].Higher yield of biosurfactant production was performed by *Aeromonas sp.* at the temperature of 40°C[31].*Bacillus licheniformis* was resistant till a temperature of 50 ^{0}C and *Arthrobacter protophormiae* grow on extreme temperatures of 30 to 100 ^{0}C[34].

7.3.5 Effect of pH

pH plays an important role in maintaining acidic or alkaline conditions during biosurfactant production. Based on the nature of microorganism the optimal pH for biosurfactant production will differs. *Cunninghamella echinulata*had maximum growth in pH 4, 7, and 9[33].*Candida lipolytica* had optimal growth at pH 7 [16]. At pH 8, biosurfactant produces maximum emulsification index of 70% was observed in the growth of *Aeromonas sp.*[31].*Bacillus sp.*produces higher biosurfactant at pH 11 and low biosurfactant production was observed in pH 9[35]. *Bacillus licheniformis* was resistant till the pH range of 4.5 and 9.0 during biosurfactant production [34].

7.3.6 Effect of incubation period

Usually biosurfactants are produced in the log phase of growth curve, thus incubation period plays a vital role in efficient production. Lin *et al.* reported that *Alcaligenes piechaudii CC-ESB2* showed maximum growth atan incubation period of 7 days with 160 rpm [2].*Cunninghamella echinulata*had maximum growth within 96 h [33].*Candida*

*lipolytica*showed maximumgrowth at 72 h for biosurfactant production [16]. Ilori *et al.* noted that growth of *Aeromonas sp*. was maximum at 8th day and cell population gradually decreased after 10th day [31].*Bacillus sp.* showed maximum growth after 40 h for biosurfactant production [35].

7.3.7Effect of salinity

Salinity is one of the important parameter that affects the cellular activity and thereby the biosurfactant production. However, in most of the studies, the biosurfactant production was not affected up to 10% (w/v) salt concentration and also slight reduction in CMCs was noted[36].Khopade et al. noticed that slight changes were observed in biosurfactant activity while increasing the sodium chloride concentration upto 9% (w/v), although 80% of biosurfactant activity was retained at higher salt concentrations[37]. The isolate *Alcaligenes piechaudii CC-ESB2* was able to grow even at 7% NaCl concentration [2].*Cunninghamella echinulata*had salinity level of 15 % [34]. 5% salinity was optimal in the growth of *Aeromonas sp.,* and noted that the emulsification index gradually decreased after 5% [31].*Bacillus licheniformis*was resistant upto 50 and 25 gL^{-1} NaCl and Ca concentrations [34].The factors influencing biosurfactant production are discussed in the table 2.

Table 2 Production parameters for biosurfactant production

Organism	Carbon source	Nitrogen source	Temperature (C)	pH	Incubation period(hr)	Reference
Aeromonas sp	Glucose, crude oil, diesel	Soybean (5% NaCl)	40	7,8	96	[31]
Alcaligenes piechaudii CC-ESB2	Diesel oil	(NaCl 7%)	37		168	[2]
Bacillus megaterium	2% crude oil, peanut oil cake, waste motor lubricant oil	-	38	8	168	[30]
Candida lipolytica	Used Vegetable oil waste	6% Soya bean residue, 1%glutamic acid	27	7	50	[16][32]
Candida sphaerica	2.5% ground-nut oil refinery residue, glucose	5.0% corn steep liquor (5% NaCl)	30	10	144	[16]
Cunningamel la echinulate	4% corn steep liquor	3% soybean oil waste (7% NaCl)	30	7.1	96	[33]
Fusarium sp.	corn steep liquor	soybean oil waste	30	7	192 to 360	[33]

7.4 Production in fermentation

Thavasi*et al.* reported in paper that a 3L fermentor was used with a working volume of 2.1 L to grow *Bacillus megaterium*. The process was carried out with a culture conditions of pH 8, temperature 38^0C, salinity 30%, 2% substrate concentration (crude oil, waste motor lubricant oil and peanut oil cake),6 mg l^{-1} dissolved oxygen concentration and 350 rev min^1[30]. Erlenmeyer flask was used as a reactor to culture *Aeromonas* in MSM broth with crude oil as sole carbon source grown at room temperature with 120rpm shaking for 10 days at pH 7. Trace element solution was aseptically introduced. The biosurfactant was recovered from the supernatant by cold acetone precipitation [31]. Experiments carried out in continuous, fed-batch, immobilized cells on calcium arginate and classical stirred tank reactor showed low productivity yields of less than 500 mg $l^{-1}h^{-1}$ [4]. For rhamnolipids production, fed batch operation under nitrogen limitation conditions gave maximum production [10]. The biosurfactant production studies were carried out in CSTR and SBR. Soil contaminated with diesel oil was used. Diesel removing efficiency in SBR was 96% but only 75% for CSTR [3]. However researchers developed an integrated chemostat system for production of rhamnolipids from *Pseudomonas aeroginosa*, by combining a STR with two membrane modules. First module would retain the bacterial cells and second prevents froth formation by enhancing gas exchange this helps to yield maximum productivity of 545 mg $l^{-1}h^{-1}$ [38].

7.5 Purification of biosurfactants

Biosurfactants are extra cellular metabolites produced by microorganisms. The purification of biosurfactants is done in the case of commercial products. Usually, 100% pure surfactant is hard to achieve. Thus, partially purified surfactant emerged. Culture of 72h growth is filtered using a Whatman no. 1 filter paper, then centrifuged at 5000 rpm for 20 min. Then the supernatant was concentrated by freeze drying following twice extraction using chloroform in the separatory funnel at 28°C [16], [39]. Using acid precipitation method, surfactant can be removed. The culture medium was centrifuged at 5000 rpm for 15 min. The supernatant was precipitated by adding con. HCl and again centrifuged at 5000 rpm for 15 min. The surfactant was extracted with dichloromethane dissolved in water,which is filtered through Whatman No. 4 filter paper [40]. From the culture, 5µl is taken centrifuged for 20 min and the extract was collected with chloroform and methanol (2:1 v/v). The solvents are evaporated using rotary evaporator the residue is purified using silica gel column and extracted using chloroform ethanol mixture. The resultant mixture is dialysed using distilled water and freeze dried using lyophilizer, the dry powder is weighed and stored [30]. Nowadays,partial purification was carried out by acetone precipitation method using supernatant collected from the culture medium. In this method the sample was dried and debris were removed with acetone and rediluted with distilled water [31].

8. Applications of biosurfactant

Biosurfactants are widely used in many industries such as food industries, pharamaceutical industries, refinery industries, metallurgical industries, agricultural sectors, chemical industries, paper industries and in environmental protection. Theyhave a promising application and also benefits economy. Numerous techniques such as physical, chemical and biological are available to degrade hydrocarbons and remediating the heavy metals. Physical and chemical methods are not suitable for treating because it creates further pollution. Nowadays, biological method such as bioremediation with the help of biosurfactant is found to be an effective and promising tool in treating the hydrocarbons and heavy metals. Moreover this methods havingmore benefits, such as less toxic to living beings, no chances for biomagnifications and less expensive way of production.

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8.1 Polyaromatic hydrocarbon degradation

PAH present in form of pericondensed and catacondensed. Pericondensed has one or more internal carbon core whereas Catacondensed has no internal carbon core. The possible ways where PAH's gets in atmosphere as pollutant are by heat and power generation by using fossil fuels, gasification/liquefaction of fossil fuels, coke production, carbon-black production, catalytic cracking, asphalt production, coal-tar/coal-tar-pitch production, wood-treatment processes, refining/distillation of crude oil, crude-oil-derived products, wood-preservative (e.g.creosote/anthracene-oil) production, transportation, fuel/oil storage, open burning (tyres/refuse/coal etc.), landfill/waste dumps, disposal and incineration [41].

Usage of petroleum products are more in many countries it creates PAH pollution. PAH's causes environmental pollution and it must be degraded. Physical and chemical methods are high cost and prone to incur air & water pollution. Hence, biological methods are preferred for treating the PAH [42]. The primary stage in microbial remediation of PAHs is the action of dioxygenase, which replaces two carbon atoms by oxygen atoms in the benzene rings forming dihydroxylated intermediates. Later they undergo ring cleavation to form TCA- cycle intermediates [5]. Mineral nutrient, sawdust, hay and compost are been chosen as amendments in heavy oil remediation [43]. In laboratory scale analysis, various PAH's like benzene, toluene, ethylbenzene and xylene were added to the culture medium along with inoculum. After 120 h of incubation at 25 ^{0}C, spectrometric analysis is carried out (OD_{600}). Many researcher concludes that bacteria with higher hydrophobicity, emulsification index and surface tension reduction were effective degradation [2].

Now, the environmentalists have put emphasis on Microbial enhanced oil recovery (MOER), because the chemical and mechanical methods recovered only 20-30 % of the oil where as microbial extraction efficacies are more [4]. The key mechanisms for MOER arelowering oil mobility ratio, porosity & permeability modification, emulsification,alteration inmicrobial metabolic pathways, alteration in wettability and using sodium bicarbonate, modification of interfacial forces andsolubilisation of oil [10].

8.2 Heavy metal remediation

High risk and cost of disposal of pollutant in landfills and incineration methods leads to new methods of disposal [44]. Heavy metal like Cu, Cd, Pb and Zn contaminated soils are considered as most hazardous. They don't drain into the ground water nor gets absorbed by soil, but they getaccumulated in plants and animals cause health problems. Remediation of heavy metals involves two mechanisms, either immobilizing it to a tight solid matrix disabling them to migrate or making them to migrate into a liquid phase by desorption and solubilising in washing solution [45].By the use of natural minerals like zeolite, clinoptilolites, nano structured fullerens, carbon nanotubes, and adsorption of heavy metals from wastewater are efficient in treating but the cost is high [46].

Several reactions govern the heavy metals they include adsorption, precipitation,complexation, demethylation, methylation, reduction andoxidation. Remediation of metal-contaminated soils is usually carried out by "in situ" i.e. stabilisation and "ex-situ" i.e. extraction. Mostly, a technique called composting (stabilisation technique) is adopted, where complexing, absorbing and precipitation takes place. Composting usually depends on pollution level, compost types and soil types [43].Biosurfactants are non-toxic and it is the potential way of treating heavy metal that are added to washing water by process of solublization, dispersal and desorption of contaminants [44]. Hassen *et al.*observed that biosurfactant produced by *Pseudomonas aeruginosa* having ability to remove 46% of chromium [47]. Dursun*et al.*noted that biosurfactant produced by *Aspergillus niger*having removal efficiency of 21-36% of chromium and 13-88 % of lead [48]. Roane*et al.*found that 36% of cadmium removal using biosurfactant producing *Bacillus strain* H9 [49]. Ravindran*et al.* observed that lipopeptide biosurfactant produced by *Bacillus* sp. MSI 54 having ability remediate 97.73% of Pb, 75.5% of Hg, 99.93% of Cd and 89.5% of Mn [50]. Dahrazma *et al.*

investigated that rhamnolipid biosurfactant can able to remove heavy metals from sediments was up to 37% of Cu, 13% of Zn, and 27% of Ni without adding any additives [51].

8.3 Biomedical approaches

Biosurfactants have good antibacterial, antifungal and antiviral properties that can be used in therapeutic purposes, it's action was reported against various gram-positive and gram-negativepathogenic and semi-pathogenic bacteria like *Alcaligenes faecalis,Acetobacter calcoaceticus, Bordetella bronchiseptica, Bacilluspumilis, Escherichia coli, Enterobacter cloacae, Citrobacter freundii, Klebsiella aerogenes ,Mycobacterium smegmatis, Micrococcus flavus, Proteus mirabilis, Proteus vulgaris*and *Serratia marcescens.*The antibiotic ability of biosurfactant molecules (lipopeptides) to self interact and form a micelle aggregate inside a lipid membrane. Biosurfactants have an antiviral activity and it helps to inactivation of retrovirus and herpes virus, this action was due to the physico-chemical interactions with the virus envelope.

Biosurfactants are used as washes to prevent biofilm formation of pathogens that helped maintaining sterility in operation theatres and surgical instruments.Biosurfactants having probiotic activity this helps to preventing and curing of bacterial vaginosis,vaginal *candidiasis*and lowers urinary tract infections. Anti-inflamatory and immune-modulatory activities is also reported that they induce immunity against numerous toxic antimetabolites [17]. Anticancer activity was also been reported that few extracellular microbial glycolipids induce cell separation in the place of division in human promyelocytic leukemia. Also in PC 12 cells and MEL (Maurine eruthroleukemia cells) the movement of acetylcholine esterase was improved along with invasion of cell cycle [34].

9. Conclusion

In this review paper, the classification and types of biosurfactants are consolidated into simple concept for the researchers to understand easily. This paper summarizes the types,sampling & isolation methods for biosurfactant producers, screening techniques and factors influencing the biosurfactant production are discussed. Moreover this paper focuses on the properties, production and purification methodologiesinvolved for biosurfactant. Atlast the applications of biosurfactant in heavy metal removal, hydrocarbon degradation and in biomedical approaches are well discussed.

Reference

1. Mulligan, Catherine N., Raymond N. Yong, and Bernard F. Gibbs. "On the use of biosurfactants for the removal of heavy metals from oil-contaminated soil." *Environmental Progress & Sustainable Energy* 18.1 (1999): 50-54.
2. Lin, Ta-Chen, et al. "Hydrocarbon degrading potential of bacteria isolated from oil-contaminated soil." *Journal of the Taiwan Institute of Chemical Engineers* 40.5 (2009): 580-582.
3. Mulligan, Catherine N., and BERNARD F. Gibbs. "Types, production and applications of biosurfactants." *Proceedings-Indian National Science Academy Part B* 70.1 (2004): 31-56.
4. Fiechter, Armin. "Biosurfactants: moving towards industrial application." *Trends in biotechnology* 10 (1992): 208-217.
5. Samanta, Sudip K., Om V. Singh, and Rakesh K. Jain. "Polycyclic aromatic hydrocarbons: environmental pollution and bioremediation." *TRENDS in Biotechnology* 20.6 (2002): 243-248.
6. Lars Järup; Hazards of heavy metal contamination, *British Medical Bulletin*, Volume 68, Issue 1, 1 December 2003, Pages 167–182.
7. Zajic, J. E., William Seffens, and Chandra Panchal. "Biosurfactants." *Critical Reviews in Biotechnology* 1.2 (1983): 87-107..

8. Rahman, Pattanathu KSM, and Edward Gakpe. "Production, characterisation and applications of biosurfactants-Review." *Biotechnology* (2008).
9. Pacwa-Płociniczak, Magdalena, et al. "Environmental applications of biosurfactants: recent advances." *International journal of molecular sciences* 12.1 (2011): 633-654.
10. Varjani, Sunita J., and Vivek N. Upasani. "Critical review on biosurfactant analysis, purification and characterization using rhamnolipid as a model biosurfactant." *Bioresource technology* 232 (2017): 389-397.
11. Shoeb, Erum, et al. "Classification and industrial applications of biosurfactants." *Academic Research International* 4.3 (2013): 243.
12. Zajic, J. E., William Seffens, and Chandra Panchal. "Biosurfactants." *Critical Reviews in Biotechnology* 1.2 (1983): 87-107.
13. Liu, Boqun, et al. "Purification and characterization of biosurfactant produced by *Bacillus licheniformis* Y-1 and its application in remediation of petroleum contaminated soil." *Marine pollution bulletin* 107.1 (2016): 46-51.
14. Ibrahim, Wael M., Asad F. Hassan, and Yahia A. Azab. "Biosorption of toxic heavy metals from aqueous solution by *Ulva lactuca* activated carbon." *Egyptian Journal of Basic and Applied Sciences* 3.3 (2016): 241-249.
15. Kiran, G. Seghal, et al. "Optimization and production of a biosurfactant from the sponge-associated marine fungus *Aspergillus ustus* MSF3." *Colloids and Surfaces B: Biointerfaces* 73.2 (2009): 250-256.
16. Diniz Rufino, Raquel, et al. "Characterization and properties of the biosurfactant produced by Candida lipolytica UCP 0988." *Electronic Journal of Biotechnology* 17.1 (2014): 6-6.
17. Fracchia, Letizia, et al. "Biosurfactants and bioemulsifiers biomedical and related applications–present status and future potentials." *Biomedical science, engineering and technology*. InTech, 2012.
18. Walter, Vanessa, Christoph Syldatk, and Rudolf Hausmann. "Screening concepts for the isolation of biosurfactant producing microorganisms." *Biosurfactants*. Springer, New York, NY, 2010. 1-13.
19. Sobrinho HBS, Rufino RD, Luna JM, Salgueiro AA, Campos-Takaki GM, Leite LFC, et al. Utilization of two agroindustrial by-products for the productionof a surfactant by Candida sphaerica UCP0995. Process Biochem 2008;43:912–7.
20. Lotfabad, Tayebe Bagheri, et al. "An efficient biosurfactant-producing bacterium Pseudomonas aeruginosa MR01, isolated from oil excavation areas in south of Iran." *Colloids and Surfaces B: Biointerfaces* 69.2 (2009): 183-193.
21. Zita, Anna, and Malte Hermansson. "Determination of bacterial cell surface hydrophobicity of single cells in cultures and in wastewater in situ." *FEMS Microbiology Letters* 152.2 (1997): 299-306.
22. Hermansson, Malte, et al. "Hydrophobic and electrostatic characterization of surface structures of bacteria and its relationship to adhesion to an air-water interface." *Archives of Microbiology* 131.4 (1982): 308-312.
23. Kjelleberg, S., and T. A. Stenström. "Lipid surface films: interaction of bacteria with free fatty acids and phospholipids at the air/water interface." *Microbiology* 116.2 (1980): 417-423.
24. Souza, Ellen Cristina, Thereza Christina Vessoni-Penna, and Ricardo Pinheiro de Souza Oliveira. "Biosurfactant-enhanced hydrocarbon bioremediation: an overview." *International biodeterioration & biodegradation* 89 (2014): 88-94.
25. De, Sourav, et al. "A review on natural surfactants." *RSC advances* 5.81 (2015): 65757-65767.
26. Mulligan, Catherine N. "Environmental applications for biosurfactants." *Environmental pollution* 133.2 (2005): 183-198.
27. Poremba, Knut, et al. "Marine biosurfactants, III. Toxicity testing with marine microorganisms and comparison with synthetic surfactants." *Zeitschrift für Naturforschung C* 46.3-4 (1991): 210-216.

28. Flasz, A., et al. "A comparative study of the toxicity of a synthetic surfactant and one produced by *Pseudomonas aeruginosa* ATCC 55925." *Medical science research* 26.3 (1998): 181-185.
29. Rosenberg, Elizabeth, et al. "Transcriptional activation and protein binding by two regions of the rat surfactant protein A promoter." *American Journal of Physiology-Lung Cellular and Molecular Physiology* 277.1 (1999): L134-L141.
30. Thavasi, R., et al. "Production and characterization of a glycolipid biosurfactant from *Bacillus megateriumu*sing economically cheaper sources." *World Journal of Microbiology and Biotechnology* 24.7 (2008): 917-925.
31. Ilori, M. O., C. J. Amobi, and A. C. Odocha. "Factors affecting biosurfactant production by oil degrading Aeromonas spp. isolated from a tropical environment." *Chemosphere* 61.7 (2005): 985-992.
32. Youssef, Noha H., et al. "Comparison of methods to detect biosurfactant production by diverse microorganisms." *Journal of microbiological methods* 56.3 (2004): 339-347.
33. Andrade Silva, Nadielly R., et al. "Biosurfactant-and-bioemulsifier produced by a promising *Cunninghamella echinulata* isolated from caatinga soil in the northeast of Brazil." *International journal of molecular sciences* 15.9 (2014): 15377-15395.
34. Roy, A. "Review on the Biosurfactants: Properties, Types and its Applications." *J Fundam Renewable Energy Appl* 8.248 (2017): 2.
35. Cooper, David G., and Beena G. Goldenberg. "Surface-active agents from two Bacillus species." *Applied and environmental microbiology* 53.2 (1987): 224-229.
36. A. S. Abu-ruwaida, I. M. Banat, S. Haditirto, A. Salem and M. Kadri, "Isolation of Biosurfactant-Producing Bacteria Product Characterization and Evaluation," Acta Biotechnol., vol. 11, no. 4, pp. 315–324, 1991.
37. A. Khopade, R. Biao, X. Liu, K. Mahadik, L. Zhang, and C. Kokare, "Production and stability studies of the biosurfactant isolated from marine *Nocardiopsis* sp. B4," *Desalin.*, vol. 285, pp. 198–204, 2012.
38. Gruber. T., PhD thesis, University of Suttgart, Germany, 1990.
39. Cirigliano, Michael C., and George M. Carman. "Isolation of a bioemulsifier from Candida lipolytica." *Applied and Environmental Microbiology* 48.4 (1984): 747-750.
40. Meenakshisundaram, M., and M. Pramila. "Detoxification of Heavy Metals Using Microbial Biosurfactant." *Int. J. Curr. Microbiol. App. Sci* 6.3 (2017): 402-411.
41. Wilson, Susan C., and Kevin C. Jones. "Bioremediation of soil contaminated with polynuclear aromatic hydrocarbons (PAHs): a review." *Environmental pollution* 81.3 (1993): 229-249.
42. Wang, Xin, et al. "Bioelectrochemical stimulation of petroleum hydrocarbon degradation in saline soil using U-tube microbial fuel cells." *Biotechnology and Bioengineering* 109.2 (2012): 426-433.
43. Chen, Ming, et al. "Bioremediation of soils contaminated with polycyclic aromatic hydrocarbons, petroleum, pesticides, chlorophenols and heavy metals by composting: applications, microbes and future research needs." *Biotechnology Advances* 33.6 (2015): 745-755.
44. Mulligan, Catherine N., Raymond N. Yong, and Bernard F. Gibbs. "On the use of biosurfactants for the removal of heavy metals from oil-contaminated soil." *Environmental Progress & Sustainable Energy* 18.1 (1999): 50-54.
45. Zhan, HuiYing, et al. "Study on removal efficiencies and mechanism of heavy metal from sewage-irrigated soils by saponin compared with commonly used washing agents." *Int. J. Sci. Eng. Res* 3 (2012): 461-469.
46. Burakov, Alexander E., et al. "Adsorption of heavy metals on conventional and nanostructured materials for wastewater treatment purposes: A review." *Ecotoxicology and environmental safety* 148 (2018): 702-712.
47. Hassen A, Saidi N, Cherif M & Boudabous A, Effect of heavy metals on *Pseudomonas aeruginosa* and *Bacillus thuringiensis*, *Bioresour Technol*, 65, 1998, 73-82.

48. Dursun A Y, Ulsu G, Cuci Y & Aksu Z, Bioaccumulation of copper (II), lead (II) and chromium (VI) by growing *Aspergillus niger*, *Process Biochem*, 38, 2003, 1647-1651.
49. Roane T M, Josephson K L & Pepper I L, Dual-bioaugmentation strategy to enhance remediation of contaminated soil, *Appl Environ Microbiol*, 67, 2001, 3208-3215.
50. Ravindran A, Sajayan A, Priyadharshini GB, Selvin J and Kiran GS (2020) Revealing the Efficacy of Thermostable Biosurfactant in Heavy Metal Bioremediation and Surface Treatment in Vegetables. Front. Microbiol. 11:222.
51. Dahrazma, Behnaz, and Catherine N. Mulligan. "Investigation of the removal of heavy metals from sediments using rhamnolipid in a continuous flow configuration." *Chemosphere* 69.5 (2007): 705-711